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MOLECULAR CHARACTERIZATION OF OIL CONTENT OF SOYBEAN

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ABSTRACT

Soybean is an important leguminous crop having 40-44% protein & 20% oil. In soybean, presence of different type of fatty acid in different quantity is major problem associated with genetic constitution of crop species. The aim of present research work was to review content of different types of fatty acid in cultivars of soybean at primary level. Thirteen cultivars release by M. K. V., Parbhani were studied. Fourteen soybean specific SSR primers were used to reveal genetic diversity of soybean cultivars.

Thirteen cultivar of soybean have different percentage of oil, MAUS-1 genotype of soybean is having highest percentage of oil (22.34%) followed by JS-93-05 (21.44%). Fourteen primers had shown diversity in a range of 54.5% to 83.3% having different banding pattern. From nutrition point of view, low linolenic, low palmatic & high oleic acid contain is desired in soybean oil. The primary objective of this study is to analyze different binding pattern of different fatty acid specific primer at molecular level.

Overall study revealed that SSR marker can reveal the quantitative trait loci (QTL) governing the trait oil content of soybean. the diversity revealed by molecular characterization of soybean had shown that different cultivar had different fatty acid content.

From the results of this study, a further study can be undertaken to correlate polymorphism of this data with quantitative estimation of different fatty acids (linolenic, palmatic, oleic)**.**

MAUS-1 has highest oil content but found some major linolenic acid specific band missing in the DNA profile. From the nutrition point of view low linolenic acid content is desired in genotypes of soybean. MAUS-1 can be the target germplasm of further study on quantitative estimation of linolenic acid due to its height oil content.

KEYWORDS: Glycine Max, Simple Sequence Repeat (SSR), Marathwada Agriculture University Soybean (MAUS)

INTRODUCTION

Soybean [*Glycine max (L.)Merr.*] Is a leguminous crop of family Leguminoceae with subfamily Papillionadae having origin in China. India's kharif season crop output for 2013-14 is expected to witness higher soybean production because of increased acreage under cultivation. The area under soybean cultivation in the country has increased from 9.33 million hectares in 2011-12 to 11.25 in 2013 million hectares. "Nature's versatile" crop, soybean is rich source of proteins and oil. As Indian diet is deficient in proteins, both in quality and quantity, soybean which is a rich source of proteins and oil, can be considered as poor man's meat. Soybean is the largest oilseed crop of the world contributing 58 % in total oilseed production.

Soybean oil is composed of five major fatty acids, which are synthesized in the seed during development. In general, wild-type soybeans contain 10–12% palmatic acid, 3-4% stearic acid, 20–25% oleic acid, 50–55% linoleic acid, and 8–10% linolenic acid. Soybean oil has industrial uses and is also a source of vegetable oil for human consumption. For these divergent applications, a modified fatty acid composition profile is sometimes desirable.

Molecular markers have provided novel powerful tool for breeders to search new source of variation and to investigate genetic factors controlling quantitatively inherited traits. The term microsatellite was coined by Litt & Lutty (1989) simple sequence repeats (SSR) also known as microsatellites are present in the genomes of all eukaryotic these are ideal DNA Markers for genetic mapping & population study because of their abundance. These are tandemly arranged repeats of mono- ,di-, tri- .tetra-, & penta- nucleotides with different length of repeats motifs (AT, CT, TA, ATT, AAT, TAT, TAA in soybean).This SSR length polymorphism at individual loci are detected by PCR using locus specific flanking region primers where the sequence is known.(H. S. Chawala, 2000).

The importance of high oleic (80%), low linolenic (10-12), low palmatic acid (8-10) content is to increase the shelf life of soybean & also reduce the oxidation of oil. The aim of this study is to explore molecular variation at oleic acid, linolenic acid and palmatic acid content of soybean germplasm of Marathwada Krishi Vidyapeeth, Parbhani.

MATERIALS AND METHODS

The present research work entitled, Molecular characterization of oil content of soybean [*Glycine max (L.) Merr.*] was carried out at Department of Post Harvest and Food Biotechnology, Vilasrao Deshmukh College of Agricultural Biotechnology, Latur constituent college to M.K.V., Parbhani. SSR is a PCR based molecular method, most widely used in the germplasm characterization and for measurement of genetic diversity in a vast array of crops, now a day (Patterson, 1996).

Estimation of oil is carried out by NMR method by using MultiQuanta Analyzer, by using RI analysis software. A total of thirteen genotypes of soybean (G*lycine max.*) viz, MAUS-1, MAUS-2, MAUS-32, MAUS-47, MAUS-61, MAUS-71, MAUS-61-2, MAUS-81, MAUS-158, MAUS-162 , JS-93-05, JS-97-52, JS-335 were collected from Soybean Research Station, M.K.V., Parbhani.

Methods

Estimation of Oil by NMR Method

Estimation of oil is carried out by NMR method by using MultiQuanta Analyzer, by using RI analysis softwar given by Gerhard Knothe James A. Kenar. (2004).

- Firstly maintained the temperature of instrument up to 40 ^{o}C (due to this it give proper result).
- Than weighed the sample on weighing balance which is connected with instrument.
- Put weighed sample in to NMR analyzer.
- Reading from RI analysis software has been taken.

Isolation of Genomic DNA and Purification

DNA was extracted from the leaves samples of soybean by method of cetyl trimethyl ammonium bromide (CTAB), Saghai-Maroof (1984).

PCR Optimization and SSR Amplification

Reagents and their Stock Solutions

Regent's Stock Solutions

dNTP's 10 mM

$MgCl_2$ 25 mM

Taq DNA polymerase 5 unit/µl

PCR buffer 10 x

Primers (Forward & reverse) 10 pm/µl

TBE 10 x

Ethidium bromide 10 mg/ml

SSR Amplification

Gene specific primers related with oleic, liolenic and palmatic acid will be used in this study. A master mix in sterile double distilled water having all the above mentioned compounds in required quantities will be prepared. Amplifications will be performed in a thermal cycler using the following cyclic parameters.

Specific SSR primers were used as: Fad3A(L), Fad3B(L), FAD6(L), AAPTla(L), Satt350(L), Satt634(L) Satt599(L), Satt200(L), FAD-1A(O), FAD2-1B(O), Satt634(O), Satt684(P), Satt368(P), Satt454(P).

Initial Denaturation 94^{O}C for 4 minutes

Denaturation 94^{O}C for 1 min

Annealing temperature 35^{O}C for 1min Primer Extension } 40 cycles

Final Extension 72^{O}C for 10 min

Final hold 4^{O}C

Agarose Gel Electrophoresis:

The amplified products will be resolved on 1.5 % Agarose gel in 1 x TAE buffer at 100V for 31/2 hour. The gel will be stained in ethidium bromide. After electrophoresis the gel will be carefully taken out of the casting tray and photograph will be taken on a gel documentation system.

Scoring and Data Analysis

Each amplification product will be considered as molecular marker and scored across all samples. Bands will be scored as present (1) or absent (0). Missing and doubtful cases will be scored as (9). Molecular weight of the bands can be estimated by using 1kb DNA ladder as standards. The data will be used for similarity based analysis using the programme NTSYS-Pc (Version 2.02) Dice's similarity coefficients (F') will be calculated using the programme SIMQUAL. Similarity coefficients can be used to construct UPGMA (unweighted pair group method with average) to generate dendrogram.

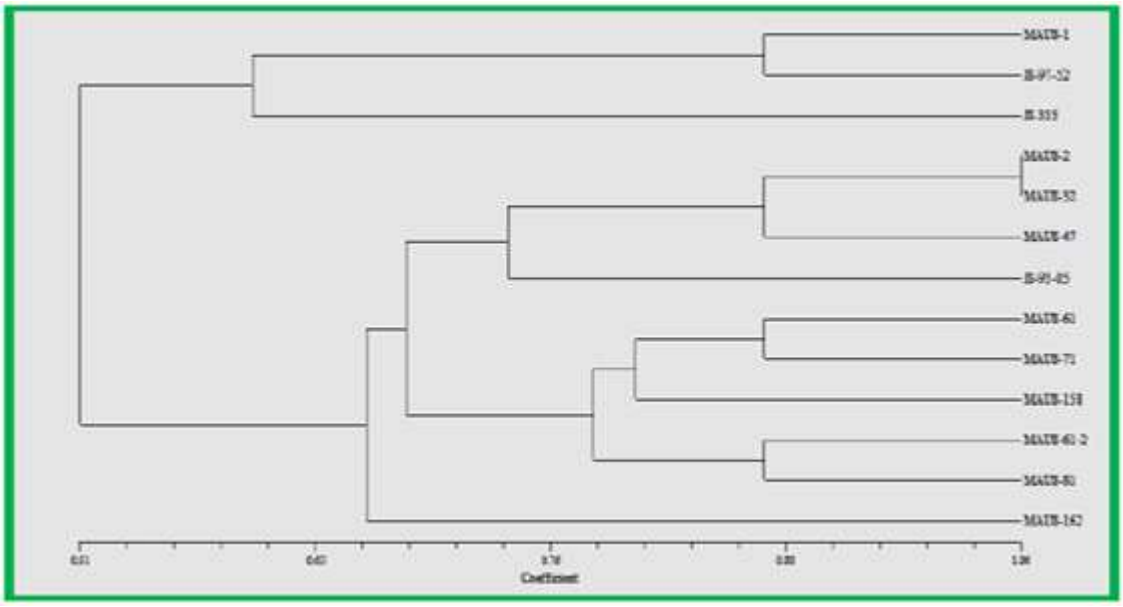

Figure 1: **Dendrogram Generated by UPGMA Analysis based on SSR Data Showing Relationship among 13 Soybean Enotype**

The polymorphic percentage of the obtained bands will be calculated by using following formula.

Polymorphic % = (no. of polymorphic bands /Total bands) X 100

RESULTS AND DISCUSSIONS

Result of Estimation of Soybean

Estimation of oil is the process to know per cent oil present in the oil seed crop, total 13 cultivar of soybean were used to estimate the percentage of oil. Estimation of oil was done by NMR method.

MAUS-1 genotype of MKV is having highest percentage of oil (22.34%) followed by JD-93-05. MAUS-47 germplasm of MKV contained lowest percentage of oil (17.22%) followed by JS-97-52(19.42%).

SSR Analysis

Different soybean specific SSR primers were used to screen soybean cultivar having different characteristics & oil content. Many loci were similar in shattering and non-shattering cultivars of soybean using SSR primers. The PCR amplification result showed that 14 SSR primers generated 117 bands.

Out of amplified 117 bands, 19 bands were polymorphic and 98 bands were monomorphic amongst 13 genotypes. The result showed that 14 SSR loci exhibited 61.9% polymorphism among 13 genotypes studied. In case of percentage polymorphism highest percentage of polymorphism was recorded in SSR primer Fad-3B(L), Satt350(L), Satt634(O).

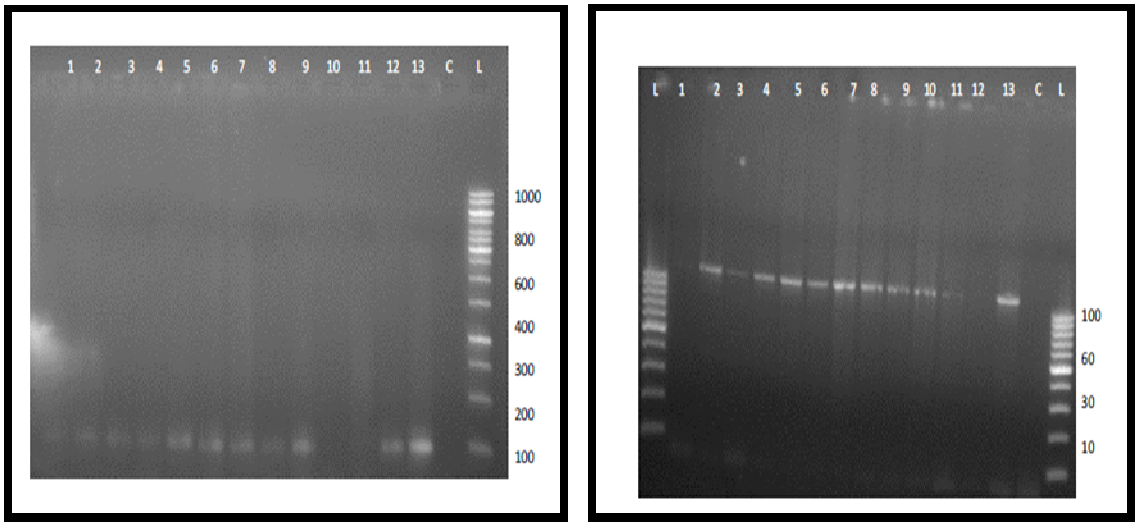

Figure 2-3: SSR Profile of Soybean Genotype with Primer FAD-3A and Satt-368

Similarity Co-efficient Analysis

Genetic relationship between 13 genotype of soybean were determined on the basis of presence of band was scored as (1) and absence as (0) was subjected to **NTSYS-pc analysis** computer software to calculate similarity among them. 100% maximum similarities were recorded. Further 48% average minimum similarities were recorded. The value of similarity coefficient ranged from 0.26 to 1.00. The highest average similarity coefficient value was calculated from MAUS-47(0.74) and MAUS-1 was represented lowest average dissimilarity coefficient value (0.53). The average genetic similarity coefficient value was 0.66.

	MAUS-1	MAUS-2	MAUS-32	MAUS-47	MAUS-61	MAUS-61-2	MAUS-71	MAUS-81	MAUS-158	MAUS-162	JS-93-05	JS-97-52	JS-335
MAUS-1	1.00												
MAUS-2	0.60	1.00											
MAUS-32	0.60	1.00	1.00										
MAUS-47	0.46	0.86	0.86	1.00									
MAUS-61	0.46	0.60	0.60	0.73	1.00								
MAUS-61-2	0.46	0.60	0.60	0.73	0.73	1.00							
MAUS-71	0.33	0.73	0.73	0.86	0.86	0.86	1.00						
MAUS-81	0.46	0.73	0.73	0.73	0.73	0.86	0.86	1.00					
MAUS-158	0.33	0.73	0.73	0.86	0.73	0.73	0.86	0.73	1.00				
MAUS-162	0.33	0.60	0.60	0.73	0.60	0.73	0.73	0.73	0.60	1.00			
JS-93-05	0.46	0.73	0.73	0.73	0.46	0.60	0.60	0.60	0.60	0.60	1.00		
JS-97-52	0.86	0.73	0.73	0.60	0.60	0.46	0.46	0.60	0.46	0.46	0.46	1.00	
JS-335	0.53	0.53	0.53	0.53	0.66	0.53	0.53	0.53	0.53	0.53	0.26	0.66	1.00
AVERAGE	0.53	0.72	0.72	0.74	0.67	0.68	0.72	0.71	0.68	0.63	0.60	0.62	0.56
MAXIMUM	0.86	0.86	0.86	0.86	0.86	0.86	0.86	0.73	0.60	0.60	0.86	0.86	0.66
MINIMUM	0.33	0.53	0.53	0.53	0.46	0.46	0.46	0.53	0.46	0.46	0.26	0.66	0.26

Figure 3: **Dice Similarity Coefficient of 13 Genotype of Soybean**

SUMMARY AND CONCLUSIONS

Summary

The present study was undertaken with the objective of assessing the genetic diversity of soybean genotypes, varietal characterization and variation at molecular level based on Linolenic, oleic, palmatic acid content. 14 SSR primers were used for genetic diversity analysis of 13 soybean genotypes. The summary of molecular work is as follows:

- Thirteen genotype of soybean were estimated by NMR method to know % oil.

- Out of 13 genotype of soybean MAUS-1 had highest percent of oil up to 22.34%.where as MAUS-47 had lowest percent of oil up to 17.22.
- Total, 117 bands were generated. Out of these bands, 19 were polymorphic and 98 bands were monomorphic.
- The monomorphic bands per SSR locus were 7.53 on an average whereas; 1.46 per SSR locus in case of polymorphic bands.
- The highest similarity coefficient value was shown by MAUS-47, i.e.0.74
- The lowest average similarity coefficient value was shown by MAUS-1 i.e. 0.53.
- Total average genetic similarity coefficient value for 13 soybean cultivars was 8.58.
- MAUS-1 germplasm has highest percentage of oil (22.34%) which is correlated with Satt368 primer specific for palmatic acid in which it give unique banding pattern
- JS-335 genotype has oil percentage of oil (20.42%) which is correlated with FAD2-1B specific to oleic acid it give unique banding pattern
- MAUS-47 cultivar content lowest percentage of oil (17.22%)which is correlated with linoleic acid specific primer i.e. FAD-6 & FAD-3A,
- MAUS-162 has oil percentage (20.43%) which is correlated with primer Satt599 specific for linoleic acid, in banding pattern MAUS-162 give unique banding pattern in Satt599.
- Satt350 (L) is linolenic acid specific SSR primer (soybase.org) it show (100bp) in all cultivar.
- Satt454 (P) is palmatic acid specific SSR primer (soybase.org) it show (50bp) in all cultivar.

CONCLUSIONS

Based on the above mentioned results, it can be concluded that the SSR marker can be used to trace the polymorphism at genetic level in soybean cultivars. In present study, the trait content of linolenic acid, oleic acid, palmatic acid were distinguishing factor for soybean cultivars. Thirteen soybean cultivars were distinguished as different percent of oil content. In 13 soybean cultivar MAUS-1 had highest percent of oil (22.34%) where as MAUS-47 had lowest percent of oil (17.22%).

Fourteen SSR primers were used for genetic diversity analysis of soybean cultivars. SSR primer FAD-1A had amplified a band of molecular weight 500 bp in only JS-97-52cultivars which is specific for only oleic acid content. SSR primer FAD-1B had amplified a band of molecular weight 500 bp in only JS-93-05 cultivars which is specific for only oleic acid content. SSR primer Satt599 had amplified a band of molecular weight below 100 bp in only MAUS-162 cultivars which is specific for only linolenic acid content. SSR primer Satt368 had amplified a band of molecular weight 100 bp in only MAUS-1cultivars which is specific for only palmatic acid content. Satt350 (L) is linolenic acid specific SSR primer it show (100bp) in all cultivar.Satt454 (P) is palmatic acid specific SSR primer it show (50bp) in all cultivar. It can be concluded that this DNA band may be a part of Quantitative Trait Loci (QTL) which governs the content of different percent of oil of soybean cultivar.

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